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EFFECTS OF AGE ON CALCIUM TRANSPORT ACTIVITY OF SARCOPLASMIC RETICULUM IN FAST- AND SLOW-TWITCH RAT MUSCLE FIBRES

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SUMMARY

1. The calcium transport activity of the sarcoplasmic reticulum (SR) was measured in chemically skinned single fast- and slow-twitch muscle fibres from young (3 months) and old (23–24 months) rats. Contractile properties, the myosin heavy chain (MHC) composition and enzyme histochemical features were studied in relation to the SR characteristics.

2. In fast-twitch single motor units, the contraction time of the isometric twitch increased ($P < 0.001$) from 13 ± 1 ms in young animals to 18 ± 2 ms in old ones. In the slow-twitch soleus, the contraction ($P < 0.001$) and half-relaxation ($P < 0.05$) times increased from 30 ± 5 and 45 ± 10 ms, respectively, in the young animals to 43 ± 3 and 55 ± 4 ms in the old ones. The proportion of slow-twitch (type I) fibres increased ($P < 0.05$) with age in the soleus from 92 ± 6 to $98 \pm 2\%$ and the proportion of fast-twitch fibres (type IIA) decreased ($P < 0.01$) from 6 ± 5 to $0 \pm 0\%$.

3. The Ca^{2+} accumulation capacity (an index of SR volume), the rate of Ca^{2+} uptake and the fractional rate of SR filling (an estimate of the specific activity of the Ca^{2+} pump) were decreased by 18 ($P < 0.05$), 32 ($P < 0.01$) and 32% ($P < 0.001$), respectively, in the old fast-twitch muscle fibres. In the slow-twitch muscle fibres, on the other hand, no significant age-related changes were observed in the Ca^{2+} transport activity of the SR. Thus, ageing exerts a differential influence on SR volume and function in fast- and slow-twitch fibres.

4. It is concluded that an age-related impairment of intrinsic SR function and a decrease in SR volume are probable factors underlying the decreased speed of contraction of fast-twitch muscle fibres in old age. In the slow-twitch soleus, on the other hand, one or more other mechanisms are responsible for the age-related decrease in the speed of contraction. The loss of fast-twitch muscle fibres in old soleus is one mechanism, but not the dominant one.

INTRODUCTION

During ageing, a decreased speed of contraction, i.e. a prolonged contraction time of the isometric twitch, has been observed in various mammals, including man (Gutmann, Hanzlikova & Vyskočil, 1971; Campbell, McComas & Petito, 1973;

Caccia, Harris & Johnson, 1979; Belanger, McComas & Elder, 1983; Davies, White & Young, 1983; Larsson & Edström, 1986; Edström & Larsson, 1987; Quinlan, Iaizzo, Lambert & Gronert, 1989; Ansved & Larsson, 1989). It has been speculated that this reduction in the speed of contraction in old age, which is seen before the senile muscle wasting becomes manifest (Larsson & Edström, 1986), may be due to an age-related loss of fast-twitch muscle fibres or to changes in the properties of the contractile material irrespective of the muscle fibre type, or both (Campbell *et al.* 1973; Newton & Yemm, 1986). The results from recent studies of fast- and slow-twitch rat skeletal muscles and single motor units have shown that the reduced speed of isometric contraction during ageing is primarily due to alterations in the contractile properties of both fast- and slow-twitch motor units, and that the age-related decrease in the number of fast-twitch muscle fibres is of lesser importance in this respect (Larsson & Edström, 1986; Edström & Larsson, 1987; Ansved & Larsson, 1989).

Various factors in the excitation–contraction coupling may play a role in the regulation of the maximum speed of shortening in individual motor units. The capacity of the SR for calcium release and recapture, on the one hand, and the composition and specific activity of fast and slow isoforms of the myofibrillar proteins, on the other hand, are the two key factors that determine the maximum speed of contraction (e.g. Brody, 1976; Heilmann & Pette, 1979; Dulhunty & Valois, 1983; Kugelberg & Thornell, 1983). Therefore, the intrinsic function and the volume of the SR as well as the myosin heavy chain (MHC) composition in fast- and slow-twitch muscle fibres were studied in the present series of experiments in order to improve the understanding of the mechanisms underlying the age-related decrease in the speed of contraction. A preliminary account of this work has been presented elsewhere (Larsson, Edström & Salviati, 1989).

METHODS

The study was carried out on male albino rats of the same strain (Wistar), fed *ad libitum* with standard laboratory food and tap water. Animals that were sick or moribund or which displayed gross pathological organ changes were excluded from the study. The animals were anaesthetized with an intramuscular injection of fentanyl-fluanisone (0·2–0·3 ml kg^{-1}) followed by pentobarbitone (30 mg kg^{-1}) administered intraperitoneally. The anaesthesia was kept at a similar level between animals by following noxic stimuli and reflex responses. If necessary, additional intraperitoneal injections of pentobarbitone were given (20–30% of the initial dose) during the experiments. Rats of the Wistar strain have a mean length of life of approximately 24 months and may live for about 36 months (Institute for Laboratory Animal Resources, 1981). In order to avoid unpredictable effects on skeletal muscle in very old age, such as those of extreme obesity, disease and disuse, we chose to study old rats that had not yet reached an advanced age. The rats were divided into a young (3 months, $n = 10$) and an old (23–24 months, $n = 10$) group.

Physiological technique. With the animal under anaesthesia, the L4 ventral root to the fast-twitch tibialis anterior muscle (TA) was exposed by laminectomy, transected proximally and maintained in a mineral oil pool formed by the skin around the incision over the vertebral column. The skin over the lower part of the left hindlimb and the fascia overlying the muscle were removed, the distal part of the muscle was freed and the plantar flexor muscles were denervated by transecting the motor nerves (Edström & Kugelberg, 1968; Kugelberg & Lindegren, 1979). The animal was then placed in the prone position on a steel plate heated to maintain the body temperature. The dissected limb was rigidly fixed in a bath filled with circulating, thermostatically controlled (36 °C) mineral oil.

Single motor units in the TA were functionally isolated by microdissection of the L4 ventral root. The criterion was an all-or-none twitch response to finely graded current pulses 0·2 ms in duration.

The tendon of the TA was attached to a strain gauge (UC 2; Statham Instruments, Inc., Oxnard, CA, USA) and the length of the muscle was adjusted to obtain maximum twitch force. The contraction time, half-relaxation time and peak force of the isometric twitch were measured before and after a short tetanus, 0·5 s in duration and with a stimulation frequency corresponding to 150 Hz.

The muscle fibres of the unit were depleted of glycogen by stimulation with trains of twenty impulses with a frequency of 100 Hz repeated once a second until the tetanic force had decreased to near zero. The stimulation was then terminated and the unit was stimulated with one impulse train every tenth second until the force had almost recovered. This sequence repeated three to five times (for details see Kugelberg & Lindegren, 1979; Edström & Larsson, 1987).

The contractile properties were studied in the fast-twitch TA and not in the extensor digitorum longus (EDL), since the latter muscle has not been found suitable for studies of single motor units with the present experimental set-up and since a large amount of reference data from single motor units in the rat TA muscle are available in our laboratory (e.g. Kugelberg & Lindegren, 1979; Edström & Larsson, 1987; L. Larsson, T. Ansved & L. Edström, unpublished observations). This reference material was used in the present study to get fast-twitch single motor units in young (3–6 months) animals that had enzyme-histochemical properties identical to and had tetanus forces similar to those in the old rats, so as to permit a reliable comparison of contraction and half-relaxation times and of the maximum rate of increase in tetanus force without risking bias due to differences in twitch or tetanus forces.

The tendon of the soleus was then attached to the strain gauge (UC 2; Stratham Instruments Inc.) and a load cell accessory (UL 4–10; Statham Instruments Inc.) and the length of the muscle was adjusted to obtain maximum twitch force. The motor nerve to the soleus was stimulated and the contraction time, half-relaxation time and peak force of the isometric twitch were measured before and after a short tetanus, 2 s in duration and with a stimulation frequency corresponding to 100 Hz.

Histological technique. After each experiment the TA and soleus were gently dissected free from surrounding tissue and clamped at approximately the *in situ* length. The muscles were then weighed, frozen in freon chilled with liquid nitrogen and stored at −80 °C until processed further. The muscle was cut perpendicular to its longitudinal axis into 10 μm thick cross-sections with a cryotome (−20 °C) at the greatest girth (TA) or at the motor point (soleus).

The muscle fibres in the motor units (TA) were mapped as unstained fibres in PAS (periodic acid–Schiff)-stained sections and identified in the subsequent sections stained for myofibrillar ATPase after alkaline and acid pre-incubations and classified as type I, IIA and IIB (for references see Pool, Donselaar & Griep, 1978; Edstrom & Larsson, 1987). The soleus cross-sections were stained for myofibrillar ATPase at pH 9·4, after 55 min of formaldehyde fixation at 4 °C and after acid pre-incubation at pH 4·35, and classified as type I, IIA and IIC (for references see Edström & Larsson, 1987). The total number of fibres of each type was counted on magnified photomicrographs of whole muscle cross-sections and the relative number of each type was calculated. These latter counts and calculations were only performed in the soleus and not in the EDL or TA, since it has been shown that the total number of fibres, the fibre size and fibre type proportions are not significantly affected by age within the 3–24 month age span in these two fast-twitch muscles, and they have similar fibre type proportions (Larsson & Edström, 1986; Edström & Larsson, 1987).

Chemical skinning. The fast-twitch EDL and the slow-twitch soleus muscles have a simple, parallel fibre arrangement and an optimal size (muscle weight approximately 200–250 mg) and are therefore suitable for chemical skinning and microdissection (the TA is too large (900–1400 mg) and has a complex fibre arrangement).

The EDL and soleus were dissected free from surrounding tissue, tied to a wooden stick and stretched to 110–120% of their slack length. The muscle specimens were then chemically skinned for 24 h by exposure to a 'skinning' solution containing 5 mM-K_2EGTA, 170 mM-potassium propionate, 2·5 mM-Na_2K_2ATP, 2·5 mM-magnesium propionate and 10 mM-imidazole buffer, pH 7·0, as described by Salviati, Sorenson & Eastwood (1982*a*). Twenty-four hours of this treatment at 0 °C has little effect on SR morphology but leads to extensive alterations of the plasma membrane (Eastwood, Wood, Bock & Sorenson, 1979). The muscle specimens were then stored at −20 °C until analysed in the same skinning solution made up in 50% glycerol.

Ca^{2+} uptake measurements. At least five single fibres, subsequently classified as type I (soleus) and type II (EDL) according to their myosin heavy chain (MHC) composition, were isolated from each muscle under a dissection microscope and mounted in a chamber containing a 'relaxing' solution (170 mM-potassium propionate, 5·0 mM-Na_2K_2ATP, 2·5 mM-magnesium propionate, 5 mM-EGTA, 10 mM-imidazole propionate, pH 7·0). Fibres were stretched to 120–130% of their slack length

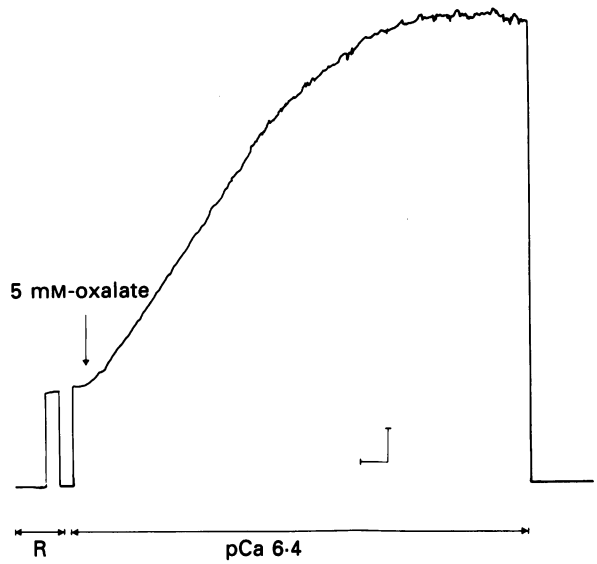

Fig. 1. Time course of the light-scattering increase due to calcium oxalate accumulation. The muscle fibre was first exposed to a relaxing solution (R) and then to the standard loading medium (pCa 6·4) to obtain the scattering by the relaxed fibre. The vertical deflection during the relaxation indicates the closing of a shutter in the light path. Oxalate was added 30 s after the fibre was exposed to the standard solution. During Ca^{2+} uptake, the increase in scattering as calcium oxalate precipitates inside the SR is proportional to the amount of Ca^{2+} accumulated. Vertical and horizontal bars represent 20 mV and 1 min, respectively.

between two clamps and the solution was vigorously stirred. The fibres were incubated in the standard loading solution containing 170 mM-potassium propionate, 2·5 mM-magnesium propionate, 5·0 mM-Na_2K_2ATP, 5·0 mM-K_2EGTA, 2·15 mM-Ca^{2+} and 10 mM-imidazole propionate, pH 7·0. The concentration of free Ca^{2+} was 0·39 μM (pCa 6·4); in calculating this an apparent association constant of $1{\cdot}919 \times 10^6$ was used for Ca-EGTA (Orentlicher, Brandt & Reuben, 1977). For Ca^{2+} concentrations higher than pCa 6·4, fibres were stretched to 180% of their slack length to avoid interference in the light-scattering measurements caused by actin–myosin interactions. In the presence of 5 mM-oxalate, active Ca^{2+} transport leads to formation of calcium oxalate crystals in the lumen of the SR and to a progressive increase in light scattering by the fibre (Fig. 1). The relative increase in light scattering is proportional to the increase in the Ca^{2+} content and the attained plateau level of the light scattering represents the maximum capacity for Ca^{2+} uptake and provides an index of the total SR volume (Sorenson, Reuben, Eastwood, Orentlicher & Katz, 1980). The rate of loading of the SR, normalized to the maximum capacity of the fibre, i.e. the SR volume, gives a measure of the percentage of the SR volume filled per minute (fractional rate of filling of the SR, for details and references see Salviati *et al.* 1982*a*; Salviati, Betto, Danieli Betto & Zeviani, 1983). Ca^{2+} uptake by chemically skinned single muscle fibres was measured at 25 °C by monitoring the increase in light scattering in the same incubation medium after addition of oxalate (final concentration, 5 mM). Calibration of the light scattering was performed with ^{45}Ca and estimates of V_{max} and K_m for the dependence of the calcium loading activity on the Ca^{2+} concentration were obtained as described previously (Salviati *et al.* 1982*a*).

The capacity for Ca^{2+} uptake and the maximum rate of uptake are reported to be similar in rat EDL and TA muscle homogenates (Briggs, Poland & Solaro, 1977). Both EDL and TA are fast-twitch muscles with identical fibre type proportions and in neither of the muscles are any significant changes in fibre type proportions or fibre size observed between 3 and 24 months of age (Larsson & Edström, 1986; Edström & Larsson, 1987). The SR properties in the chemically skinned fibres from these two muscles were therefore considered comparable.

Determination of myosin heavy chain composition. In each animal, nine or ten chemically skinned muscle fibres from the EDL and the soleus were incubated at room temperature overnight in a solubilization solution (2·3% (w/v) SDS, 5% (w/v) 2-mercaptoethanol, 10% (v/v) glycerol, 62 mM-Tris-HCl, pH 6·8). The MHC composition was determined by SDS polyacrylamide gel electrophoresis on 6% polyacrylamide gels, as described by Danieli Betto, Zerbato & Betto (1986). After electrophoresis the gel was stained with Coomassie Blue or silver (for details and references see Salviati, Betto & Danieli Betto, 1982*b*; Salviati *et al.* 1983).

Statistics. Means and standard deviation of the means were calculated from individual values by standard procedures. The two-tailed independent *t* test was used for intergroup comparisons unless otherwise indicated. Differences were considered significant at $P < 0{\cdot}05$.

RESULTS

In conformity with previous results of studies of this and other albino rat strains (e.g. Institute for Laboratory Animal Resources, 1981; Larsson & Edström, 1986; Edström & Larsson, 1987), the body weight increased from 323 ± 11 g in young animals (3 months, $n = 10$) to 648 ± 46 g in old ones (23–24 months, $n = 10$).

Contractile properties

In the fast-twitch TA, the muscle fibres in one single motor unit per animal were identified enzyme-histochemically in six old rats after glycogen depletion as a marker of previous muscle fibre contraction (Edström & Kugelberg, 1968; Kugelberg & Edström, 1968). The muscle fibres in these fast-twitch motor units were of the IIB type and had tetanus forces ranging between 10 and 30 g ($20{\cdot}8 \pm 6{\cdot}4$ g). These motor units were compared with six motor units from young rats (3–6 months) which had tetanus forces within the same range ($19{\cdot}3 \pm 6{\cdot}0$ g) and muscle fibres with enzyme-histochemical properties identical to those in the old ones, i.e. type IIB. The contraction time of the pre- and post-tetanus isometric twitch was significantly ($P < 0{\cdot}001$) longer in the old animals, while the other contractile properties studied showed no age-related difference (Table 1). A post-tetanic potentiation of the isometric twitch was observed in both young and old animals (Table 1).

In the slow-twitch soleus, a longer contraction ($P < 0{\cdot}001$) and half-relaxation ($P < 0{\cdot}05$) time, of both the pre- and post-tetanus isometric twitch, was observed in the old rats compared with the young ones (Table 1). The twitch force was not affected by age and a post-tetanic depression was observed in both young and old rats (Table 1).

Enzyme-histochemical properties and myosin heavy chain composition

In conformity with previous findings (e.g. Larsson & Edström, 1986; Edström & Larsson, 1987; Ansved & Larsson, 1989), a significant age-related difference in the proportion of fibre types was noted in the soleus muscle, i.e. the proportion of type I fibres was higher ($P < 0{\cdot}05$) and the proportion of type IIA fibres lower ($P < 0{\cdot}01$) in the old rats (Table 2).

TABLE 1. Characteristics of isometric twitch and tetanic contractions in fast-twitch single motor units from tibialis anterior muscle and slow-twitch soleus. Comparison of contraction time (T_c), half-relaxation time ($T_{1/2r}$) and force (P_t) of the pre- and post-tetanus twitch, and maximum rate of increase in tetanus force in young and old animals. Values are means ± S.D.

Age (months)	Motor unit muscle	n	Pre-tetanus T_c (ms)	Pre-tetanus $T_{1/2r}$ (ms)	Pre-tetanus P_t (g)	Post-tetanus T_c (ms)	Post-tetanus $T_{1/2r}$ (ms)	Post-tetanus P_t (g)	Maximum rate of increase in tetanus force (g ms^{-1})
3–6	Fast-twitch	6	13±1	14±4	5·7±2·7	13±1	14±3	6·7±2·7	0·56±0·19
23–24	Fast-twitch	6	18±2	20±10	7·7±3·3	18±2	19±9	8·3±3·1	0·65±0·26
			$P < 0·001$	n.s.	n.s.	$P < 0·001$	n.s.	n.s.	n.s.
3	Soleus	7	30±5	45±10	41·1±7·7	25·7±2·8	44·1±5·6	36·0±7·3	—
23–24	Soleus	9	43±3	55±4	42·2±5·0	39·3±2·6	50·1±3·4	37·9±4·9	—
			$P < 0·001$	$P < 0·05$	n.s.	$P < 0·001$	$P < 0·05$	n.s.	

TABLE 2. Proportion of fibre types and myosin heavy chain (MHC) composition in the extensor digitorum longus (EDL) and soleus in young and old animals. Values are means ± S.D., with ranges in parentheses

Age (months)	Muscle	n	Fibre type proportions I (%)	Fibre type proportions IIA (%)	Fibre type proportions II (%)	MHC composition I (%)	MHC composition I+IIA (%)	MHC composition IIA (%)	MHC composition IIA+B (%)	MHC composition IIB (%)
3	EDL	10	—	—	—	2±4 (0–11)	5±8 (0–22)	42±24 (0–67)	20±20 (0–56)	31±15 (11–56)
23–24	EDL	10	—	—	—	0±0 (0)	2±6 (0–22)	37±23 (11–89)	30±24 (0–70)	32±28 (0–89)
						n.s.	n.s.	n.s.	n.s.	n.s.
3	Soleus	10	92±6 (28–100)	6±5 (0–11)	2±2 (0–5)	89±15 (60–100)	10±15 (0–40)	1±3 (0–10)	—	—
23–24	Soleus	10	98±2 (92–100)	0±0 (0–1)	2±2 (1–7)	97±9 (70–100)	3±9 (0–30)	0±0 (0)	—	—
			$P < 0·05$	$P < 0·01$	n.s.	n.s.	n.s.	n.s.		

The MHCs were separated on 6% SDS gels in nine to ten fibres in each EDL and soleus muscle of the ten young and old animals. The composition of the MHCs did not differ significantly between young and old animals in either fast- or slow-twitch muscles.

SR volume, rate of Ca^{2+} uptake and fractional rate of filling

In accordance with previous findings, the SR volume was larger, the Ca^{2+}-loading rate faster and the fractional rate of filling higher in fast-twitch than in slow-twitch

TABLE 3. Ca^{2+} transport activity of the SR in fast- and slow-twitch muscle fibres. Comparison of Ca^{2+} uptake capacity rate of Ca^{2+} uptakes and fractional rate of filling in young and old animals. Values are means ± S.D.

Age (months)	Fibre type	No. of animals	No. of fibres	Ca^{2+} uptake capacity (μmol (mg fibre protein)$^{-1}$)	Rate of Ca^{2+} uptake (μmol (min)$^{-1}$ (mg fibre protein)$^{-1}$)	Fractional rate of filling
3	Fast-twitch	9	49	0·82 ± 0·30	0·077 ± 0·056	0·117 ± 0·042
23–24	Fast-twitch	9	49	0·67 ± 0·36	0·052 ± 0·043	0·079 ± 0·021
				$P < 0·05$	$P < 0·01$	$P < 0·001$
3	Slow-twitch	10	44	0·29 ± 0·20	0·017 ± 0·007	0·066 ± 0·021
23–24	Slow-twitch	9	50	0·30 ± 0·14	0·017 ± 0·007	0·061 ± 0·014
				n.s.	n.s.	n.s.

rat muscle fibres (Table 3, for references see Salviati *et al.* 1982*a*). The skinned fibres contained 123 ± 40 mg protein (ml fibre volume)$^{-1}$ and the protein content was not affected by age or fibre type. By recalculating the data in Table 3, it is shown that the fast- and slow-twitch fibres in the young animals accumulated 101 and 36 μmol Ca^{2+} (ml fibre volume)$^{-1}$, respectively. The weight of 1 ml skinned fibres was 1·16 ± 0·02 g ml^{-1} (which is close to the weight we found in fresh wet muscle, 1·23 ± 0·02 g ml^{-1}). Accordingly, the maximum accumulation of calcium oxalate by fast- and slow-twitch fibres was 87 and 30 μmol Ca^{2+} (g fibre)$^{-1}$, respectively. These values conform with the maximum accumulation of Ca^{2+} in the presence of oxalate reported in rat muscle homogenates (97 μmol in EDL and 33 μmol in soleus; Briggs *et al.* 1977). Further, the ratio of Ca^{2+} transport between EDL and soleus fibres as well as the rate of Ca^{2+} uptake at 0·4 μM-calcium concentration (Table 3) in the young animals are also very close to the values reported by Briggs and co-workers (1977) in their Fig. 1 (at 0·4 μM-Ca^{2+} concentration).

In the fast-twitch EDL muscle fibres, the SR volume (capacity for Ca^{2+} uptake) was 18% lower ($P < 0·05$) in the old rats than in the young ones. Correspondingly, there was a 32% decrease ($P < 0·005$) in the rate of Ca^{2+} pump activity in the old animals compared with the young ones (Table 3, Fig. 2). These data suggest that there is a reduction in the relaxing activity of the SR in old EDL which is mainly due to a decreased rate of Ca^{2+} pump activity. This is supported by the differences in the slopes of the linear regression curves between young and old animals (Fig. 2). These slopes give a measure of the fractional rate of filling of the SR (an estimate of the specific activity of the Ca^{2+} pump; see Methods). Thus the fraction of total SR filled

per minute was 32% lower ($P < 0.001$) in the old animals than in the young ones (Table 3).

As shown in Fig. 3, the Lineweaver–Burk plot of the fractional rates of Ca^{2+} uptake at concentrations of Ca^{2+} varying from pCa 7·0 to 5·2 is concave upward in both young and old animals, suggesting a stoichiometry greater than one for the reaction of Ca^{2+} with the pump. V_{max} (maximum initial velocity) and K_m

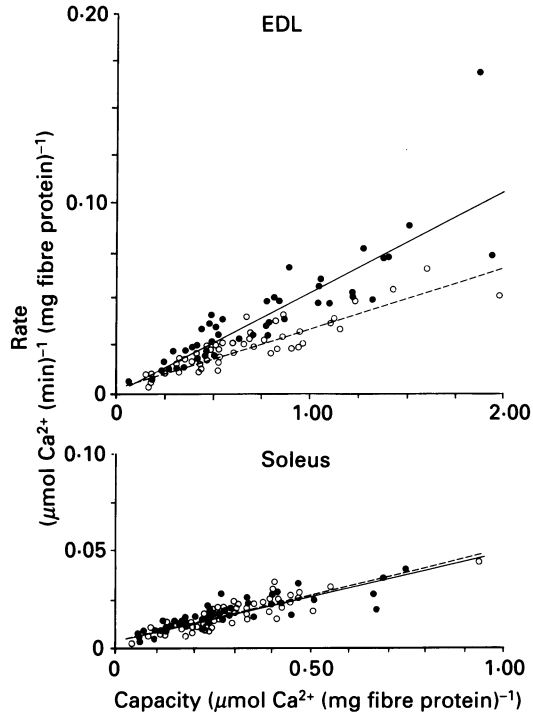

Fig. 2. Relation between SR Ca^{2+} loading rates and capacities in fast-twitch fibres from the extensor digitorum longus (EDL) and slow-twitch fibres from the soleus in young (●, continuous lines) and old (○, dashed lines) animals. The ratio of rate to capacity for each fibre gives the fractional rate of filling. In the fast-twitch muscle fibres, the slope of the regression line was 0·105 min^{-1} ($r = 0.86$) in the young animals and 0·061 min^{-1} ($r = 0.90$) in the old ones. In the slow-twitch fibres, the slope was 0·039 min^{-1} ($r = 0.87$) in young and 0·048 min^{-1} ($r = 0.89$) in old animals.

(Michaelis–Menten constant) were calculated as reported previously (Salviati *et al.* 1982*a*). The values for V_{max}, extrapolated from the linear regression curves, were similar in the young (0·195, $r = 0.97$) and old (0·202, $r = 0.99$) animals. K_m, on the other hand, was lower in the young fast-twitch fibres (0·38 μM) than in the old ones (1·07 μM, Fig. 3). Thus, the different fractional rates of filling in young and old fast-twitch EDL fibres reflected differences in the affinity of the Ca^{2+} pump, i.e. a higher Ca^{2+} pump activity was seen in young fast-twitch fibres than in old ones at low Ca^{2+} concentrations.

In the slow-twitch soleus fibres, the capacity for Ca^{2+} uptake by the SR and the Ca^{2+}-loading rates were the same in young and old animals (Table 3, Fig. 2).

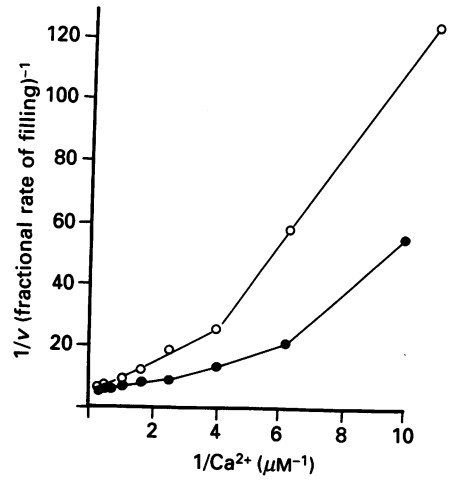

Fig. 3. Relation between rate of Ca^{2+} filling and Ca^{2+} concentration. Ten extensor digitorum longus fast-twitch fibres from four young rats (●) and nine fast-twitch fibres from three old rats (○) were each exposed sequentially to several different Ca^{2+} concentrations in the presence of 5 mM-oxalate, and the maximum linear rate was recorded at each concentration. The sequence was terminated and loading was allowed to proceed to its maximal capacity when the rate of increase in light scattering reached a plateau. Values plotted are reciprocal of fractional rate of filling.

Accordingly, the fractional rate of filling was the same in young and old animals (Table 3).

DISCUSSION

The earlier observations on an age-related decrease in the speed of contraction in fast-twitch motor units and in the slow-twitch soleus muscle, and also a decreased proportion of fast-twitch fibres in the soleus muscle (Edström & Larsson, 1987; Larsson & Edström, 1987) have been confirmed in this study. Further, the present results show that age-related alterations in myosin heavy chain compositions/fibre type proportions are not dominant factors underlying the decreased speed of isometric contraction in old age in either fast- or slow-twitch motor units or muscles. In fast-twitch motor units, the decreased speed of contraction is primarily related to alterations in the SR. The observation by Kugelberg & Thornell (1983) of a close correlation between the volume of the SR (i.e. the terminal cisternae) and the contraction time of single motor unit isometric twitches, thus indicates that the age-related reduction in the SR volume could be the factor underlying the decreased speed of contraction in old age. However, the rate of Ca^{2+} transport depends not only on the SR volume but also on the specific activity of the Ca^{2+} pump and the present results show that the decreased speed of contraction in old age is related to both a decrease of SR content and a decrease of the pump activity.

The mechanisms underlying the age-related changes in the properties of the SR are not known, but they may be associated with an alteration in muscle protein synthesis

or catabolism induced either by a primary myogenic mechanism or by a change in motoneurone properties. The close connection between motoneurone discharge properties and the synthesis and accumulation of calcium ATPase in the SR (Jolesz & Sreter, 1981), together with the changes in motoneurone discharge properties in old age (Borg, 1981; Nelson, Soderberg & Urbscheit, 1984), lends some support to the latter alternative.

It is interesting to note that some of the age-related changes observed in contractile and SR properties in fast-twitch motor units/muscle fibres conform with results reported in myocardial cells. That is, the decreased speed of isometric contraction seen in old cardiac muscle cells from Wistar rats has been found to be coupled with a decreased rate of Ca^{2+} uptake into isolated SR vesicles (see Lakatta, 1987).

The SR volume and the intrinsic properties of the SR were not affected by age in the slow-twitch muscle fibres and another mechanism underlying the decreased speed of contraction has to be searched for. The lower proportion of fast-twitch motor units in old soleus muscle may account for part of the slowing of the whole soleus muscle response. However, this is probably not the major explanation, since an age-related decrease in the speed of contraction has also been observed in slow-twitch single motor units in the soleus (Edström & Larsson, 1987) and the loss of type II muscle fibres in the soleus precedes the decreased speed of contraction during ageing (Ansved & Larsson, 1989). The age-related loss of contractile material is more pronounced in the slow-twitch soleus than in the fast-twitch EDL or TA (Larsson & Edström, 1986; Edström & Larsson, 1987). Increased stiffness of the parallel elastic component, as indicated by the increased resting tension at L_0 (optimum muscle length) in old soleus (Larsson & Edström, 1986) – due to partial replacement of contractile material by adipose and connective tissue (Kavonen, Suominen & Peltonen, 1987; Ansved & Larsson, 1989) – is one possible reason for the observed slowing in old soleus and soleus single motor units. It is also conceivable that another factor or factors in the excitation–contraction coupling, apart from the SR properties and the MHC composition, with strong influence on the speed of contraction may become altered during ageing. However, preliminary data from chemically skinned soleus fibres show that the pCa–tension curves and the pattern of troponin C isoforms obtained by SDS gel electrophoresis are identical in young and old rats, indicating that regulatory proteins are not affected by age in slow-twitch fibres (G. Salviati & L. Larsson, unpublished observations).

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